

## REMARKS

### **I. Support for the Amendments**

Claims 1-26 were originally in the application. Non-elected claims 8-26 have been withdrawn. Claims 5 and 7 were canceled during previous Amendments.

Claims 1-4 and 6 were in the application. Claim 4 has been canceled without prejudice to its pursuit in an appropriate divisional or continuation application. Claims 1 and 3 have been amended. No new matter has been added by virtue of these amendments. Claims 1-3 and 6, as amended, are presently in the application.

Support for amended claims 1 and 3 can be found in the original specification, figures, and claims. The amendments to claims 1 and 3 have provided the sequence identifiers in accordance with the Examiner's request. Additional support for amended claims 1 and 3 can be found, e.g., from page 10, line 12, to page 11, line 5; from page 17, line 24, to page 18, line 8; in Figures 6 and 7; and in the Examples.

Support for the amendments to the specification can be found in the original specification, figures, and claims. The specification has been amended to provide sequence identifiers in accordance with the Examiner's remarks in the Office Action and to provide sequence identifiers for sequences previously provided in the Figures (particularly in Figures 5-7). Additional support for the amendment to page 26 of the specification can be found from page 22, line 17, to page 23, line 14, of the Japanese text of PCT/JP99/02305, a copy of which is provided herewith, with the relevant portions of the text underlined and indicated by check marks in the left-hand margin.

## **II. Status of the Claims**

Claims 1-26 were originally in the application. Claims 1-26 were subject to an election/restriction requirement, and claims 1-7 were elected. Claims 8-26 were withdrawn without prejudice or disclaimer of any subject matter. Claims 4, 5, and 7 have been canceled. Claims 1-3 and 6 are presently in the application.

## **III. Nucleotide and/or Amino Acid Sequence Disclosures**

The Examiner has required amendments to the claims with respect to the sequence identifiers and has requested a revised Sequence Listing, including both a paper copy and an electronic copy.

Applicants have amended claims 1 and 3 accordingly, in addition to the appropriate portions of the specification, in response to the Examiner's remarks and to provide sequence identifiers for sequences previously provided in the Figures (particularly in Figures 5-7). Applicants have requested the Examiner to enter the revised sequence listing.

Applicants respectfully submit that the amendments to claims, specification and sequence listing place the application in condition for allowance.

## **IV. Rejection of Claims 1-4 and 6 under 35 U.S.C. §112, First Paragraph**

The Examiner has rejected claims 1-4 and 6 under 35 U.S.C. §112, first paragraph, for reasons relating to enablement. The rejection is rendered moot with respect to claim 4, which

has been canceled without prejudiced. Applicants respectfully traverse the rejection.

As amended, claim 1 recites a polypeptide having more than 50% identity with SEQ ID NO: 1 and comprising 101 conserved amino acid residues. These amino acid residues are commonly conserved in all amino acid sequences shown in Figure 7. Therefore, one of ordinary skill in the art would recognize the potential importance of these conserved amino acids, relative to the variable amino acids, with respect to the claimed nicotianamine synthase. As a result, use of part or all of the consensus sequence(s) in the present invention would not require undue experimentation on the part of one of ordinary skill in the pertinent art.

Applicants respectfully disagree for the reasons outlined *supra*, but have amended claim 1 in the interests of furthering the prosecution of the case. Support for the amendment to claim 1 can be found in Figure 7, as filed, and elsewhere. Claims 2, 3 and 6 are dependent on claim 1, and the reasoning that applies to claim 1 also applies to these claims. (Claim 4 has been canceled, rendering the rejection moot.) Applicants respectfully submit that the amendments place claims 1-3 and 6 in condition for allowance.

#### **V. Correction of Inadvertent Error in the English Translation of the Specification**

It has come to Applicants' attention that an inadvertent error occurred during the translation of the specification of PCT/JP99/02305 from Japanese into English. Applicants have amended the specification on page 26 of the English translation in order to correct the error.

First, in the translation filed with the application, the subject matter of original Example 9 was omitted, and original Example was incorrectly numbered and entitled as Example 9. Applicants have amended page 26 of the English translation to include the inadvertently omitted

text and to correct the titles of the Examples. Support for this amendment can be found from page 22, line 17, to page 23, line 14, of the Japanese text of PCT/JP99/02305. Copies of these pages are submitted herewith. The paragraph and title in question are underlined and indicated by check marks in the left-hand margin.

Second, in the text for Example 10 (as amended), the cited example number was inadvertently altered to “example 1” during the translation (see page 26, line 20). Applicants have amended page 26 to correct the cited example number to “example 9.” Support for this amendment can be found on page 23, line 8, of the Japanese text of PCT/JP99/02305. A copy of this page is submitted herewith. The line in question is underlined and indicated by a check mark in the left-hand margin.

Applicants submit that these changes are supported in the original Japanese text of the international application (PCT/JP99/02305). The present case is a U.S. National Phase Application of PCT/JP99/02305 under 35 U.S.C. §371. Applicants respectfully submit that the amendment to the specification merely corrects an inadvertent error in translation, that the error was unintentional, and that no new matter is introduced into the application as a result of the amendment to correct the translation.

Applicants respectfully request the Examiner to enter the amendment correcting the English translation, which will place the application in condition for allowance.

**CONCLUSION**

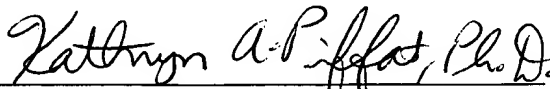
In view of the foregoing amendments and remarks, the present application is respectfully considered in condition for allowance. An early reconsideration and notice of allowance are earnestly solicited.

It is believed that all outstanding rejections have been addressed by this submission and that all the claims are in condition for allowance. If discussion of any amendment or remark made herein would advance this important case to allowance, the Examiner is invited to call the undersigned as soon as convenient.

~~It is believed that a one-month extension of time is required.~~ If a petition for an additional extension of time is required, then the Examiner is requested to treat this as a conditional petition for an additional extension of time. Although it is not believed that any additional fee, beyond the fee submitted herewith, is required to consider this submission, the Commissioner is hereby authorized to charge our deposit account no. 04-1105 should any fee be deemed necessary.

Respectfully submitted,

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ム社)に転写した。膜を0.5M チャーチリン酸 (Church and Gilbert 1984) 1mM EDTA、7% (w/v) SDS、100  $\mu$ g/ml サケ精巢DNAを含むバッファーを用いて65℃1晩でプローブとハイブリダイズした。これを40mMチャーチリン酸、1% (w/v) SDSを含むバッファーを用いて65℃10分間洗浄した。この洗浄をもう1回行った後、0.2xSSPE、0.1% (w/v) SDSを含むバッファーを用いて65℃10分間洗浄した。放射活性はイメージアナライザーBAS-2000で検出した。

結果を第9図及び第16図に示す。

#### 実施例8 (サザンハイブリダイゼーション)

オオムギとイネの葉からそれぞれゲノムDNAを抽出した。これをBamHI、あるいはEcoRI、あるいはHindIIIで断片化し、0.8% (w/v) アガロースゲル電気泳動で分離した後、ハイボンド-N<sup>+</sup>膜 (アマシャム社)に転写した。実施例7で述べた方法でハイブリダイズし、放射活性を検出した。

結果を第10図に示す。

#### 実施例9 (ポリクローナル抗体の調製)

✓ ネズミ2匹を約100  $\mu$ gの単離したニコチアナミン合成酵素を抗原として免疫した。抗原としては部分アミノ酸配列を決定したものと同一試料を用いた。1回目の免疫時には完全フロイントアジュバント、2回目以降は不完全フロイントアジュバントを用いた。4回免疫した後、全採血を行い、血清を-80℃で保存した。

#### ✓ 実施例10 (ウエスタンブロット解析)

トリクロロ酢酸とアセトンを用いて全タンパク質を抽出した (Damerval et al. 1986)。植物体を液体窒素中で粉状になるまで粉碎し、10% (w/v) トリクロロ酢酸、0.1% (v/v) 2-メルカプトエタノール (2-mercaptoethanol) を含むアセトンと混合した。-20℃で1時間静置してタンパク質を沈殿させた後、16,000xg 30分遠心して沈殿を回収した。沈殿を0.1% (v/

v) 2-メルカプトエタノール (2-mercaptoethanol) を含むアセトンに懸濁し、  
-20℃で1時間静置してタンパク質を沈殿させた後、16,000 x gで30  
分遠心して沈殿を回収した。沈殿を減圧乾燥した後、試料バッファー (9.5 M  
尿素 (urea), 2% (w/v) トリトン-X-100 (Triton X-100), 5%  
(v/v) 2-ME) に溶かし、16,000 x g 10分遠心して上清を得た。  
これに含まれるタンパク質をSDS-PAGE、あるいは変性2次元電気泳動  
(O'Farrell 1975) で分離した後、PVDF膜に転写した。この膜に対して、実  
施例 9 で調製した抗ニコチアナミン合成酵素抗体を1次抗体、西洋ワサビペルオ  
キシダーゼ結合抗マウスIgG (H+L) ヤギ抗体 (和光純薬) を2次抗体とし  
て、ジアミノベンジジンで発色しウエスタンブロット解析を行った。

結果を第12図に示す。SDS-PAGEは12.5%アクリルアミドスラブ  
ゲルで行った。100 μgのタンパク質を泳動した。根については200 μg、  
葉については500 μgのタンパク質を泳動した。

#### 実施例11 (RT-PCR)

シロイヌナズナから全RNAを抽出し、その1 μgを鋳型としてEZ rTth RNA PCR  
キット (パーキンエルマー社) を用いてRT-PCRを行った。プライマーはAtNAS1、  
AtNAS2、AtNAS3それぞれに特異的なものを用いた。結果を第18図に示す。

#### 産業上の利用可能性

本発明の組換えベクターを用いて種々の細胞を常法に従って形質転換すること  
ができ、得られた形質転換体を用いてニコチアナミドを大量に製造することがで  
きる。これらの方法は当業者に知られている方法により行うことができる。

また、本発明の遺伝子を用いて、植物、好ましくはイネ科植物の品種の改良を  
行うこともできる。特に、鉄分が欠乏している土壌においても生育できる品種に  
改良するために、本発明の遺伝子を利用することができる。